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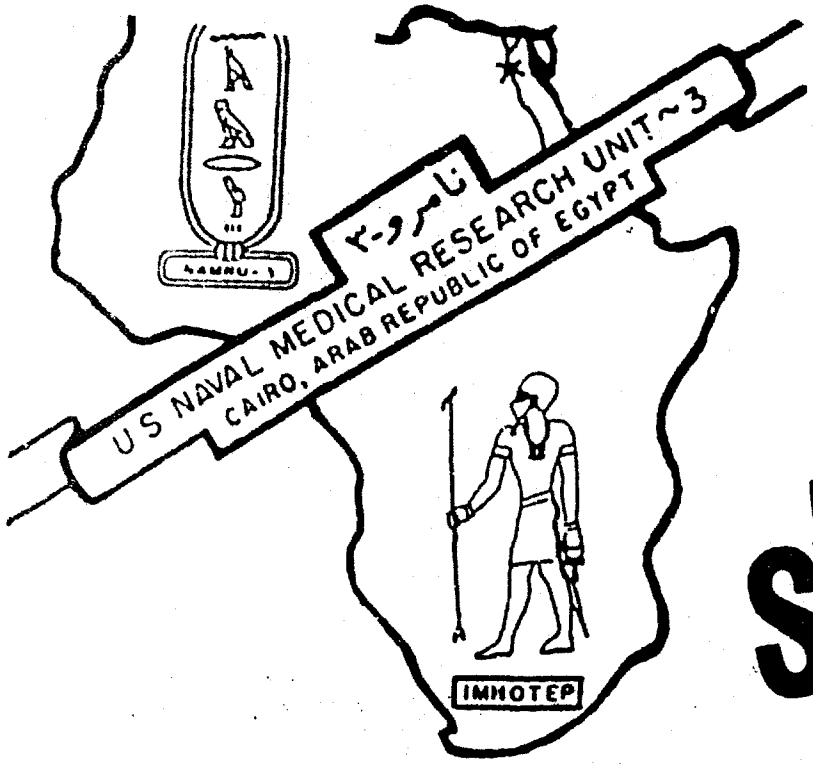

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FIELD TRIAL OF 1% NICLOSAMIDE AS A TOPICAL ANTIPENETRANT TO SCHISTOSOMA MANSONI CERCARIAE

BY

Renon R. Abu-Elyazeed, John K. Podgore, Noshy S. Mansour, and Michael E. Kilpatrick

U.S. NAVAL MEDICAL RESEARCH UNIT NO. 3
(CAIRO, ARAB REPUBLIC OF EGYPT)
PSC 452, BOX 5000
FPO AE 09835-0007

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A randomized, double-blind, placebo-controlled field trial of a topical anti-penetrant lotion, 1% niclosamide, applied daily to the upper and lower limbs of farmers occupationally exposed to Schistosoma mansoni cercarial-infested water, was conducted in the Nile Delta to assess its safety and efficacy in preventing reinfection. Farmers aged 18-40 years were treated to cure their S. mansoni infections three months prior to the onset of the trial. Subjects were randomly assigned to receive niclosamide or placebo lotion that was self-applied daily for five months. A total of 186 subjects met the inclusion criteria and completed the trial. The exposure to schistosomal-infested water occurred during routine irrigation activities from June to November 1991. Stool specimens were evaluated monthly during and for two months following the lotion application period. The subjects applying the niclosamide lotion were comparable to those applying placebo lotion in age (mean 30 years for both), total water contact (184.5 hr versus 173.8 hr), reported lotion application compliance (88% versus 92%), and reported water contact involving skin exposure other than upper and lower limbs (23% versus 27%). The schistosomal reinfection rate was lower in the niclosamide group (53.3%) compared with the placebo lotion group (71.3%), ($P < 0.02$). Increased protection might be obtained with total body application for shorter, less intense, water contact exposures.

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FIELD TRIAL OF 1% NICLOSAMIDE AS A TOPICAL ANTIPENETRANT TO *SCHISTOSOMA MANSONI* CERCARIAE

REMON R. ABU-ELYAZEED, JOHN K. PODGORE, NOSHY S. MANSOUR, AND MICHAEL E. KILPATRICK

U. S. Naval Medical Research Unit No. 3, Cairo, Egypt

Abstract. A randomized, double-blind, placebo-controlled field trial of a topical antipenetrant lotion, 1% niclosamide, applied daily to the upper and lower limbs of farmers occupationally exposed to *Schistosoma mansoni* cercarial-infested water, was conducted in the Nile Delta to assess its safety and efficacy in preventing reinfection. Farmers aged 18–40 years were treated to cure their *S. mansoni* infections three months prior to the onset of the trial. Subjects were randomly assigned to receive niclosamide or placebo lotion that was self-applied daily for five months. A total of 186 subjects met the inclusion criteria and completed the trial. The exposure to schistosomal-infested water occurred during routine irrigation activities from June to November 1991. Stool specimens were evaluated monthly during and for two months following the lotion application period. The subjects applying the niclosamide lotion were comparable to those applying placebo lotion in age (mean 30 years for both), total water contact (184.5 hr versus 173.8 hr), reported lotion application compliance (88% versus 92%), and reported water contact involving skin exposure other than upper and lower limbs (23% versus 27%). The schistosomal reinfection rate was lower in the niclosamide group (53.3%) compared with the placebo lotion group (71.3%), ($P < 0.02$). Increased protection might be obtained with total body application for shorter, less intense, water contact exposures.

Schistosomiasis remains a major public health problem of tropical and subtropical developing regions. More than 200 million persons residing in rural and agricultural areas are currently infected. It is estimated that between 500 and 600 million persons are exposed to potential schistosomal infection.[1] With the recent expansion in water development resources in the developing world, the number of people at risk is expanding.

There is no known chemoprophylaxis against schistosomiasis. The only accepted prophylactic approach to the prevention of schistosomiasis is the elimination of all direct skin contact with water infested with *Schistosoma* cercariae. Protection from schistosomal infection has been considered from four different perspectives: 1) destruction of schistosome-bearing snails, 2) elimination of cercariae by cercaricides, 3) use of protective fabrics and clothing, and 4) application of cercaricidal and/or cercarial repellents to the skin.[2] Research in the fourth area has included assessment of chemicals of the salicylanilide class. Animal tests involving mice, hamsters, and monkeys have demonstrated that one of the salicylanilide compounds, niclosamide, when dissolved in alcohol and applied to skin, was effective in preventing the penetration of schistosome cercariae.

Niclosamide (WR046234, 2',5-dichloro-4'-nitrosalicylanilide) has been used in medical therapy throughout the world for more than 30 years. It has been used for the treatment of intestinal cestode infections at a daily oral dose of two grams for seven days with no reports of serious adverse reactions.[3] It also has proven to be an effective schistosomicide and molluscicide in large-scale water treatment programs.[4] No skin irritation has been noted in humans during skin contact of niclosamide molluscicide preparations. Cutaneous application of niclosamide had no sensitizing effect in humans with photoallergy to tribromasalicylanilide.[5] Skin reactions were occasionally seen with applications of a 25% emulsion preparation of niclosamide that were proven to be due to other ingredients and not niclosamide itself.[6]

MATERIALS AND METHODS

This field trial was a prospective, randomized, double-blind, placebo-controlled study to evaluate the effectiveness and safety of 1% niclosam-

TABLE 1
Inclusion criteria

1) Absence of schistosomiasis infection as determined by three consecutive daily stool analyses at the time informed consent was obtained
2) Anticipate exposure to schistosomal-infested water during the irrigation season
3) Agree to apply the lotion daily to upper and lower limbs for six months
4) Willing to undergo regular monitoring for adherence to the protocol
5) Agree to avoid total body contact with potentially infested water for the duration of the study
6) Agree to report daily water contact
7) Agree to provide three consecutive daily stool samples every month for eight months
8) Agree to consult the study physician prior to taking any medication
9) No history of allergies to niclosamide or related compounds
10) No history of drug allergies, skin rash, seizures, or chronic medical problems
11) Absence of skin abnormalities or significant medical disorders as determined by the examining physician

ide skin lotion in preventing *S. mansoni* reinfection in Egyptian farmers who had been successfully treated for schistosomiasis. This preventive intervention strategy was endorsed by the Egyptian Ministry of Health. The community acceptance was high because of the awareness that schistosomiasis is a serious local health problem.

Study area and study population

The study was conducted in the Nile Delta region of Egypt, in three rural sites in the Abu Homos District, Beheira Governorate. Three villages, Anwar El-Mofty, Desones, and Kom El-Kanater, were selected because of the high prevalence of endemic *S. mansoni* infection detected in recent Ministry of Health surveys. The inhabitants of these villages were mostly farmers and did not differ greatly with respect to their living style and activities. Healthy male farmers aged 18–40 years who fulfilled the trial inclusion criteria (Table 1) were invited to participate in the study.

Study drug and randomization

The drug used in this study was a 1% solution of niclosamide formulated in an alcohol lotion. The placebo lotion was the same formulation (alcohol base) without the 1% niclosamide. This lotion was manufactured by Miles Pharmaceuticals (West Haven, CT). It was slightly yellow in color and was packed in 30-ml polyethylene screw-cap bottles. The lotion was approximately the consistency of insect repellent or sun-screen lotion. It was applied by removing the cap from the bottle and squeezing several milliliters into the palm. The hands were rubbed together and the lotion was then spread evenly over the upper limbs from the shoulders down to the hands and fingers. The lower limbs were then covered with lotion from the upper thighs down to the feet, including the interdigital spaces of the toes and finishing with the soles. The study medication and placebo bottles were individually labeled with one of ten code letters.

Participants were assigned identification numbers that were alpha-numeric, with the alpha component identifying the village and the numeric component corresponding to the number of participants in the particular village. The blinded, random assignment to study medication was provided by a computerized statistical model. The blinding was blocked in groups of 20. The computerized list randomized the assignment of study medication to subjects and also randomized the assignment of active drug and placebo to letter-coded bottles.

Study procedures

Approximately six months prior to the start of the study during November–December 1990, approximately 3,000 individuals were given a labeled 50-ml plastic centrifuge tube for urine and a wide mouthed screw-cap plastic container for stool and were asked to provide a urine and a stool specimen the next morning. The specimens were processed for examination within six hours of collection. Urine samples were examined by the sedimentation concentration technique.[7] Fecal samples were examined using the Kato-Katz thick smear method.[8] Treatment was carried out during January 1991, based on the results of the urine and stool samples. Each subject with a urine and/or stool specimen positive for *Schistosoma* eggs received a single oral dose

of 40 mg/kg of praziquantel under the supervision of a local Ministry of Health physician. Subjects who were not excreting *Schistosoma* eggs were excluded to ensure that only subjects with a high exposure risk were selected. Those who were excreting *S. haematobium* eggs were also excluded since they comprised less than 1% of the infected population. Twelve weeks after treatment, which was two months prior to the start of the lotion application phase of the study, three stool samples were collected on three consecutive days from all the treated subjects to identify potential candidates for the study. To detect light infections, fecal samples were examined by the more sensitive modified Ritchie concentration technique.[9] Individuals found negative for *S. mansoni* on this re-examination were considered potential subjects for the trial. A detailed medical history was obtained and a physical examination were performed on each of the potential subjects with particular attention to the presence of rashes or other skin abnormalities. From this pool of potential subjects, 600 volunteers provided written informed consent and entered the study. The application of the study lotion began in June 1991 and continued for 23 weeks.

Field evaluation

Each participant was issued seven 30-ml bottles of the study lotion per week. Study subjects self-applied the lotion daily to their upper and lower limbs. The lotion was applied in the morning before the subjects left for work in the fields. Field monitors visited the study participants' houses early in the morning three times per week on designated days and observed the lotion application, inquired if the subject applied the lotion the previous day, and collected data on the previous day's water contact. On the visit following a two-day visit interval, information was obtained regarding the previous two days. The study monitor made two additional daily visits to each participant. One visit was made during the day at the work site to make sure that there was no unprotected exposure (body areas other than the upper and lower limbs) to canal or irrigation water. The other visit was in the evening at the participant's house to ask about total water contact information for that day and to record any adverse reactions to the lotion. The monitor recorded the data on each subject's record log.

Each monitor was responsible for observing and documenting the activities of 10 study participants (five participants each day). The study subjects also were visited once a week by a field coordinator who supervised the field monitor, observed lotion application, and collected data for quality control. In addition, senior study supervisors made two to four visits to the participants per month. The principal investigators visited all participants at least once a month throughout the study duration to assess lotion application, water contact, and local or systemic reactions such as rash, itching, or wheezing. The data collected by the field monitor, the field coordinator, the senior supervisor, and the principal investigators were routinely compared and discrepancies were investigated to ensure protocol compliance. The field monitor, coordinator, or supervisor was responsible for reporting to the principal investigators when compliance was not 100%. A missed application was identified as that in which the study drug was not applied as scheduled and water contact occurred prior to the next application. Full compliance was expected from all study participants.

Laboratory evaluation

Fecal specimens were collected monthly after the start of lotion application and continued for two months after cessation of lotion application. A single collection consisted of three consecutive daily specimens. Samples of stool were examined in the Abu Homos field laboratory by the formalin ether sedimentation procedure (the modified Ritchie technique). Duplicate 10% formalin–preserved samples of 100% of stool collections three and seven months after the start of lotion application were shipped to the Department of Parasitology Laboratory, Walter Reed Army Institute of Research (Washington, DC) for repeat testing as a means of assuring quality control of laboratory analysis. Three- and seven-month specimens were considered negative when the results of the two laboratories were both negative, while they were considered positive if eggs were detected by either laboratory.

Statistical evaluation

All data entry and evaluations were completed before revealing the medication identity. The EpiInfo microcomputer programs produced by

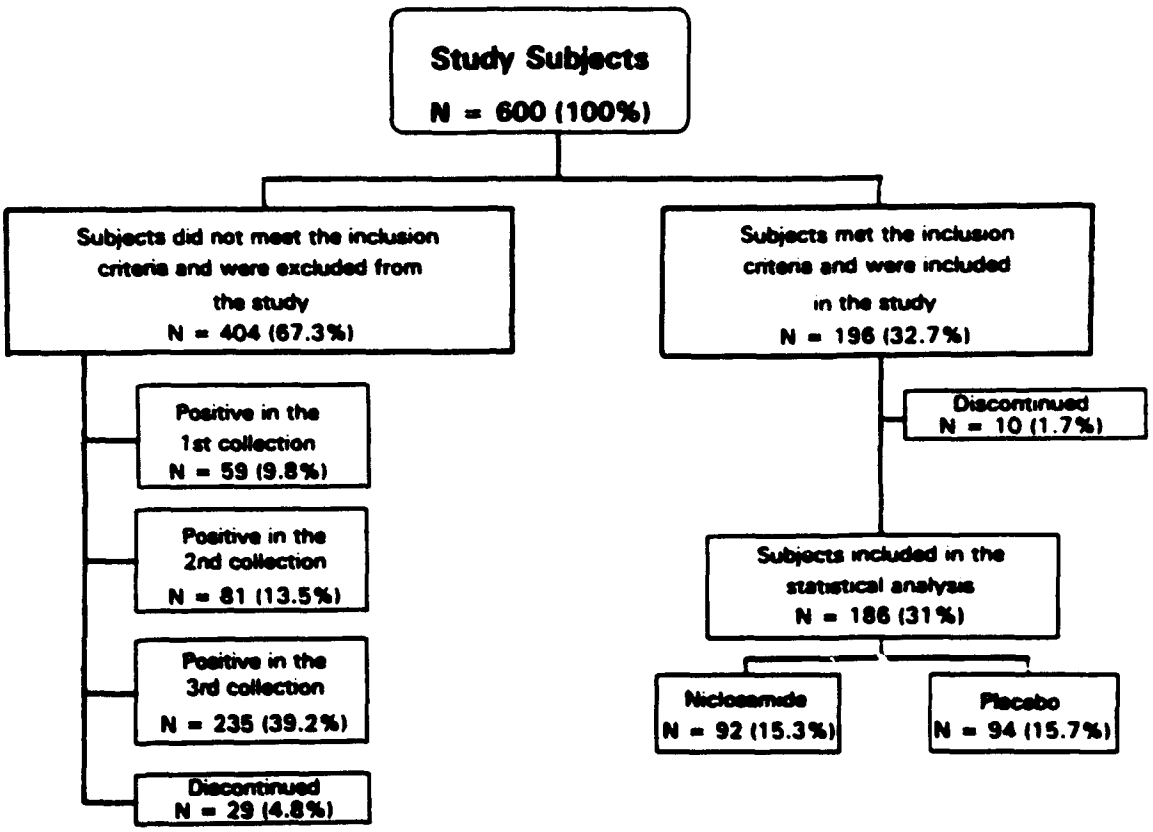

FIGURE 1. Description of the study subjects.

the Centers for Disease Control and Prevention (Atlanta, GA) and the World Health Organization (Geneva, Switzerland) were used to create questionnaires and to enter data. To ensure the accuracy of data entry, a double-entry system was used with two different operators to enter all data into two separate files. The files were then compared using a special EpiInfo program and any differences were identified so that the non-duplicate entries could be reviewed and reconciled in the two files. Evaluations included contingency table and chi-square tests for detection of differences in reinfection rates between the treatment groups. Student's *t*-test was used to test for differences of means among study groups. Odds ratios (OR) were used to estimate the protective effect and 95% confidence intervals were computed. Logistic regression analysis of data was performed using SPSS/PC V4.1 software (SPSS Inc., Chicago, IL) to adjust for possible confounding variables.

A schistosomiasis-infected subject was defined as an individual who passed one or more eggs in any stool collection four months after the start of lotion application until two months after cessation of lotion application. An infected subject detected during the first three months of lotion application was not included in the statistical analysis to eliminate the possibility of preinfec-

TABLE 2

Lotion application compliance among study groups

Study group	% compliance of lotion application			
	98–100%	90–97%	< 90%	Total
Niclosamide	81	11	0	92
Placebo	81	6	1	88*

* Six additional subjects with incomplete data were not included.

TABLE 3

Lotion application observation reported by study monitors among study groups

Study group	% observation of lotion application			
	90–100%	80–89%	< 80%	Total
Niclosamide	57	19	16	92
Placebo	58	21	9	88*

* Six additional subjects with incomplete data were not included.

TABLE 4

Mean, standard deviation, and median values of water contact in hours during the whole study period reported by study groups

Study group	No. of subjects	Mean	Standard deviation	Median
Niclosamide	92	184.5	84.5	177
Placebo	88*	173.8	85.3	152

* Six additional subjects with incomplete data were not included.

tion or incomplete eradication of infection by praziquantel.

RESULTS

Of the 600 farmers participating in the study, 404 were excluded because they did not meet the inclusion criteria (Figure 1). Of the 404 (67.3%) who were excluded, 59 (9.8%) who were positive for *S. mansoni* eggs were excluded at the first collection, 81 (13.5%) were positive at the second collection, 235 (39.2%) were positive at the third collection and 29 (4.8%) discontinued participation and did not submit stool specimens for the third collection. One hundred ninety-six (32.7%) subjects were negative for *S. mansoni* eggs in the stool at the third collection and were included in the statistical analysis. Ten of the subjects withdrew from the study; four traveled outside the study area, four subjects stopped applying the lotion for no specific reason, and two subjects submitted no stool specimens for the final stool collection. Therefore, a total of 186 subjects were included in the analysis. Ninety two (49.5%) subjects were using the 1% niclosamide lotion and 94 (50.5%) subjects were applying the placebo lotion.

The mean age of the study subjects was 30 years, which was identical in the two groups. Eighty-one subjects (88%) in the niclosamide group and 81 (92%) in the placebo group reported lotion application daily for five months with no more than one missed application (Table 2). There was no difference in compliance among the two groups. Lotion application was observed by study monitors in 90% or more of the occasions in 57 (62%) of the subjects in the niclosamide group versus 58 (66%) of the subjects in the placebo group (Table 3). The difference in observations between the two groups was not statistically significant. There were no significant differences in mean and median total canal or irrigation water contact during the entire study

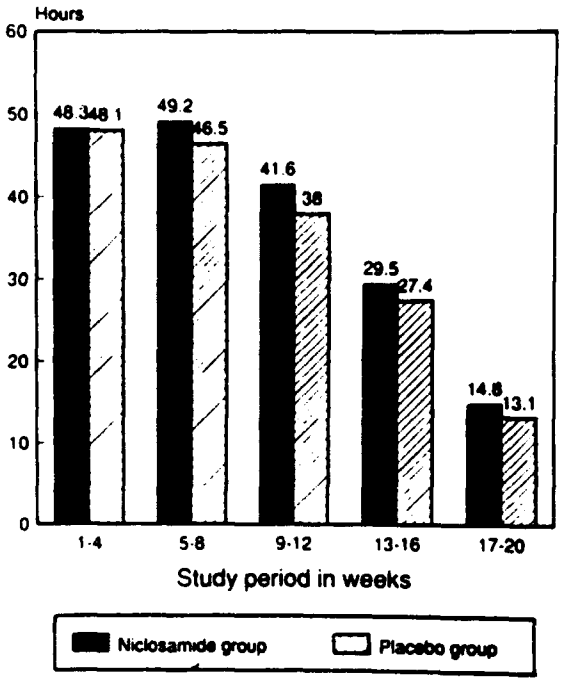

FIGURE 2. Mean water contact in hours during the study period reported by the niclosamide and placebo groups.

period between subjects receiving niclosamide and placebo lotion (Table 4). Figure 2 represents the reported water contact during the study period in the two study groups. The greatest water contact occurred during the first two months and then gradually decreased. There was no significant difference in water contact during any period between the two groups. Subjects who received niclosamide and placebo lotion were comparable in reported water contact with the skin other than the upper and lower limbs (non-lotion application areas). Twenty-one subjects (22.8%) in the niclosamide group versus 25 (27.2%) in the placebo group reported water contact other than the upper and lower limbs (Table 5).

The reinfection rate during the study was sig-

TABLE 5

Water contact episodes involving body surface areas other than the upper and lower limbs reported by the study groups

Study group	Unprotected water contact episodes			
	0	1–2	>2	Total
Niclosamide	54	17	21	92
Placebo	50	17	25	92*

* Two additional subjects with incomplete data were not included.

TABLE 6

Crude and adjusted odds ratios of Schistosoma mansoni *infection among niclosamide and placebo recipients**

Study group	*S. mansoni*		Crude odds ratio	(95% CI)	Adjusted odds ratio†	(95% CI)
Niclosamide	49	43	0.46	(0.24, 0.88)‡	0.41	(0.22, 0.77)§
Placebo	67	27	1.00		1.00	

* CI = confidence interval.
† Adjusted for reported total water contact.
‡ $P = 0.012$.
§ $P = 0.006$.

nificantly lower in the niclosamide group (53.3%) than in the placebo group (71.3%) (Table 6). A crude OR of 0.46 ($P < 0.02$) was observed for the niclosamide group. The protective effect of niclosamide lotion in preventing reinfection by *S. mansoni* became more significant (OR = 0.41, $P < 0.01$) after adjusting for total water contact, which may influence the reinfection rate.

No generalized or dermatologic side effects were reported or detected in any of the study subjects during the five-month period of daily lotion application.

DISCUSSION

The present study was conducted in one of the highest endemic areas of *S. mansoni* in the world. The daily lotion application regimen was well accepted by the farmers and a high level of compliance was observed. Farmers participating in the study were exposed to direct sun rays during their routine work in the field a minimum of four hours a day with temperatures ranging from 28°C to 40°C. No skin or systemic reactions occurred that required discontinuation of the daily lotion applications.

Although daily niclosamide lotion application was safe, well-accepted, and provided some protection against *S. mansoni* reinfection compared to the placebo, the level of protection was not enough to recommend its use for the control of schistosomiasis among agriculture workers in this highly endemic region. Limiting niclosamide lotion application only to the upper and lower limbs may have been a major reason for the low level of protection. Farmers in the study area were frequently exposed to canal water beyond the upper and lower limbs, especially during periodic canal clearance, water pump repairs, and ablution (washing of the body before Moslem prayers).

Acceptable protection might be obtained with total body application to overcome the problem of water exposure beyond the upper and lower limbs during routine work activities. Moreover, better protection might be obtained with niclosamide lotion when used by travelers, field engineers, and military forces that require less daily contact with infested water for shorter periods of time.

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Authors' address: Remon R. Abu-Elyazeed, John K. Podgore, Noshy S. Mansour, and Michael E. Kilpatrick, U. S. Naval Medical Research Unit No. 3, code: 301B, FPO AE 09835-0007.

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